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VACCINE FOR THE PREVENTION IN HUMANS OF COLDLIKE SYMPTOMS ASSOCIATED WITH THE JH VIRUS*

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The discovery of the adenoviruses^{1, 2} constituted a major advance in leading to the identification of agents responsible for common respiratory disease of presumed viral etiology in the human population. These agents have been shown to be of particular importance in military populations.³⁻⁴ However, studies from this laboratory⁵ as well as from others^{7, 8} have indicated that these agents produce little clinical disease in the civilian populations studied, although the significance of the adenoviruses in causing disease in infants and children has not yet been determined.

In agreement with the findings of Dingle and co-workers,⁹ it has been our experience that respiratory illness makes up about 70 per cent of all illnesses seen in families and various groups we have studied.⁶ No etiologic agent has as yet been isolated which accounts for the major share of such illnesses, which clinically are of the common-cold variety.

In a recent paper¹⁰ from this laboratory, evidence was presented for the isolation of a new virus (JH) which was associated with mild upper-respiratory illness in humans. In this paper we wish to report the development of a vaccine which protects individuals against the coldlike symptoms associated with the JH virus.

MATERIALS AND METHODS

Virus Isolations.—The JH virus was isolated in rhesus monkey kidney epithelial tissue cultures as described previously.¹⁰ The cultures were inoculated when 5 days old and were maintained in 199 medium (Difco) at 36.5° C., also as described earlier.¹⁰

Neutralization tests with the JH virus were carried out, using approximately 500 TCD₅₀ of the virus. A fourfold rise was considered significant. Control uninfected cultures were included in all tests. Neutralization tests were used because no reliable complement-fixation test has yet been worked out for this virus.

Examination of Children.—All children were examined daily, beginning on the third day of the outbreak, and temperatures were taken in the morning and in the evening. All children participated in this work with the written consent of their parents.

Vaccine Preparation.—The JH isolate used to prepare the vaccine was originally isolated from a child with coryza, mild sore throat, and a fever of 99.9° F. It was isolated from nasal washings inoculated into monkey kidney epithelial tissue cultures as described previously.¹⁰ The isolate had been passed ten times in monkey kidney and had a titer of 10^{7.1} TCD₅₀/0.1 ml. at its tenth passage.

The virus suspension used to prepare the vaccine was tested by the methods described in the previous paper,¹⁰ to rule out the possibility of other cytopathogenic agents being present in the JH virus suspension. The cytopathogenic effects caused by the JH virus suspension were completely inhibited if this suspension was mixed with antisera prepared against the JH virus, using other isolates of JH virus to prepare the antisera.

The infected monkey kidney epithelial cultures were harvested 8 days after the addition of the JH virus. At the same time that infected cultures were prepared, one-half of the culture tubes were left uninoculated to serve as controls and for the preparation of a placebo. Most of these tubes were harvested at the same time as the infected tubes and treated in the same manner. Ten of the control tubes were observed for 14 days. At this time no cytopathogenic effects were detectable. Two blind passages of the control suspensions were made into monkey kidney cultures, and no cytopathogenic effects were observed when these cultures were grown in 199 medium or in a medium containing 2 per cent calf serum and hydrolyzed lactalbumin or in the serum medium described previously.¹⁰ Ten tubes in each medium were observed for 14 days. These results indicate that no detectable simian viruses were present in the monkey kidney cultures used to prepare the vaccine.

After harvest of the infected cultures, the contents of the tubes were pooled and homogenized in a Waring Blendor. The suspension was centrifuged at 2,000 rpm in a small desk International centrifuge, and the supernatant fluid was then filtered through a sintered glass filter of medium porosity. The addition of antisera to the JH virus, prepared with other JH isolates, completely inhibited the vaccine preparation from causing detectable cytopathogenic effects when inoculated into monkey kidney tissue culture and observed for 10 days. Titrations showed that the virus preparation used for these neutralization tests contained approximately 10^{3.5} TCD₅₀.

Just before inactivation, the filtered JH virus preparation contained 10^{6.9} TCD₅₀.

In order to inactivate the material, a final concentration of 1:2,000 formalin was added to the solution. This preparation was held at 35° C. for 7 days, occasionally being shaken by hand. After this time the formalin-treated virus solution was dialyzed (with constant stirring) against 199 solution at 3.5° C. to get rid of free formaldehyde. After the dialysis procedure, which by itself did not significantly lower the infectivity of live JH virus, fifteen monkey kidney culture tubes containing 0.9 ml. of 199 medium were inoculated with 0.2 ml. of the dialyzed virus preparation. No detectable cytopathogenic effects were observed in 18 days, and two blind passages also failed to show evidence of cytopathogenic effects. The vaccine was stored in sterile bottles at 3.5° C.

Safety Tests.—Many safety tests were carried out. Just before the JH virus was treated with formalin, the following media were tested with aliquots of the preparation: trypticase soy agar, blood agar, tryptose agar, serum ascites agar, Lowenstein's medium, Brewer's trioglycollate broth, and Sabouraud's agar. None of the cultures showed growth of any kind when they were incubated under anaerobic and aerobic conditions at 36.5° C. or room temperature for 3 weeks. The following animals were inoculated with the virus preparation just before it was inactivated: rhesus monkeys intracerebrally and intramuscularly; rabbits intracerebrally, intraperitoneally, and intracutaneously; adult mice intracerebrally; suckling mice intracerebrally and intraperitoneally; hamsters intraperitoneally and intranasally; and guinea pigs intraperitoneally. The animals were observed for 35 days. During this time there was no fever or other sign of clinical illness. Histopathological examination of the brain and cord of the inoculated monkeys showed no sign of infection at the end of the observation period. The same safety tests were repeated on the inactivated JH virus preparation after it had been stored in the bottles for 2 days as described above. Again, all results were negative. The uninfected monkey kidney cultures were treated in the same manner as described for the infected preparation. In the safety tests at least five animals of each species were used for each vaccine or control preparation in the case of the large animals, and at least fifteen animals in the case of the small rodents.

RESULTS

Design of Vaccine Test.—Our previous experience¹⁰ with the JH virus had shown that it can be associated with mild upper-respiratory outbreaks in the human population. Therefore, fairly large groups of individuals were given either placebo or vaccine injections. In picking the individuals who received the various injections, the following procedure was followed. The names of the individuals to be inoculated were written on small slips of paper and dropped into a hat. The slips were then picked out of the hat. Every other name selected from the hat was given vaccine. The groups that were selected to be vaccinated were picked on the basis that the individuals making up these groups lived in the same building, had intimate contact with each other, and were of the same age, sex, and race. Thus, if a respiratory outbreak occurred in the group, such variables as exposure to the agent, age, sex, race, and environment could be controlled to some extent.

The physicians who examined the groups did not know which individuals in the groups received vaccine and which received placebos. The workers doing the antibody determinations against the JH virus had no idea as to the source of their

serum samples, since at that time serum samples from infections due to adenoviruses and influenza were also being tested against the JH virus as part of the general respiratory study being carried out in the laboratory.

Effect of JH Vaccine on Respiratory Outbreak.—Although a number of groups were vaccinated and given placebos, only the results of one group will be discussed in this paper, because it was the only group which experienced a respiratory outbreak associated with the JH virus.

The individuals in the group consisted of 114 children between the ages of ten and fourteen. They lived and slept in one large dormitory. This group had been broken up into three parts. All individuals in each of the groups were distributed randomly in the cottage. The 50 children making up the first group were given 1.0 ml. of vaccine intramuscularly and were given another injection 1 month later. Twenty-five children were given two injections of the control uninfected preparation, and 25 were given two injections of saline. These injections were given in the same manner as the vaccine. All the children were bled before being given their first injection. Unfortunately, because of a mixup in the bleeding schedule, the children were not bled again until 2 weeks after their second injection. They were bled again 2 weeks later. Table 1 shows that children receiving the vaccine

TABLE 1
NEUTRALIZING ANTIBODY RESPONSE TO THE JH VACCINE

Serum Specimen	Prevaccination Titer	Postvaccination Titer*
1-R	0†	1:8
3-R	1:4	1:64
5-R	0	1:16
7-R	1:8	1:128
9-R	0	1:16
11-R	1:4	1:64
13-R	0	0
15-R	0	1:64

* Sera taken 2 weeks after second injection.

† 0 means less than 1:4.

responded with neutralizing antibodies to the JH virus. Ten children given the uninfected preparation and 10 children given saline showed no increases in their neutralizing antibody titer to the JH virus. All these determinations were carried out on the sera collected 2 weeks after their second injection.

The respiratory outbreak occurred 5½ weeks after the second injection. It was characterized by coryza, mild sore throat, and fever not over 100° F., and in some cases a cough. It lasted about 2 weeks, with the great majority of the cases occurring at the beginning of the second week. The duration of illness was 4 days. The routine clinical blood examination was normal, as was a throat culture taken on any individual showing overt illness. Table 2 shows the relationship of the various clinical symptoms in the total number of overt cases that occurred during the outbreak. Three of the 26 children showing overt illness had a fever between 102 and 103 degrees for about 2 days. The other sick children had fevers of 100 degrees or less.

Tests to show the etiology of this outbreak were negative for streptococcus, adenoviruses, Coxsackie viruses, and influenza, using the methods described previously.¹⁰ However, two of eleven of the individuals showing overt disease yielded JH virus in their nasal washings. Because of the difficulty in isolating the

TABLE 2
RELATIONSHIP OF VARIOUS SYMPTOMS ASSOCIATED WITH
THE RESPIRATORY OUTBREAK

Symptoms	No. of Patients Showing Various Clinical Combinations*	No. of Patients Showing Antibody Rise to JH Virus
Coryza, mild sore throat, slight fever, cough	7/26**	7/7†
Coryza, mild sore throat, slight fever, no cough	13/26	9/13
Coryza and slight fever, no cough or sore throat	3/26	2/3
Coryza and slight sore throat, no fever or cough	3/26‡	2/3

* Denominator is total number of patients showing overt disease, and numerator is number of patients showing indicated clinical symptoms.

† Denominator is total number of patients showing various clinical relationship, and numerator is number of these showing significant neutralizing antibody rises to JH virus. Convalescent sera collected 4 weeks after illness.

‡ One of these patients received the vaccine.

** Three of these children showed fevers between 102 to 103 degrees.

JH virus with the techniques available at the present time, it is hard to assess the role of this agent in causing disease by this method. However, the serologic response to the JH virus reported in the previous paper,¹⁰ and the great specificity of this response,¹⁰ makes such a test more feasible in trying to determine the importance of this agent in causing human illness. From Table 3 it can be observed

TABLE 3
OVERT RESPIRATORY ILLNESS IN INDIVIDUALS RECEIVING
INACTIVATED JH VACCINE AND PLACEBO

Injection	No. Showing Overt Respiratory Illness	No. of Patients Showing Significant Antibody Rise to JH Virus*
Saline	11/25†	9/11
Control uninfected preparation	12/25	9/12
Vaccine	3/50	2/3

* Fourfold increase in neutralizing antibody was considered significant. Convalescent sera collected 4 weeks after illness.

† Denominator is total number of people comprising each group, and numerator is number of people developing overt respiratory disease during outbreak.

that, of 26 individuals showing overt disease, 20 showed antibody rises to the JH virus. It is felt, therefore, that the JH virus may have been responsible for this outbreak. This view is greatly strengthened by the results which showed that, of the 50 children receiving the vaccine, only 3 developed respiratory illness during this outbreak, whereas 23 children out of 50 who did not receive the vaccine developed overt disease. The sera of 3 of 15 children tested who received no vaccine and showed no overt illness during this outbreak showed significant increases in neutralizing antibody to the JH virus. Thus, on the basis of this very small sample, 20 per cent of the children experienced subclinical infection with the JH virus in this outbreak. It is realized that this number is far too small to permit any significant conclusions about the subclinical attack rate of the JH virus.

DISCUSSION

The data presented in the previous paper¹⁰ and the observation in this paper that an inactivated vaccine prepared from the JH virus will reduce the incidence of mild upper-respiratory illness about eightfold, in a respiratory outbreak with which the JH virus was found to be associated, indicate that the JH virus is capable of causing a coldlike disease in the human population.

The data indicate that an effective and safe vaccine can be prepared from inactivated JH virus against an infection which clinically is very similar to the common cold. Two major questions that remain to be answered are the length of time the protective antibodies produced by the killed JH virus vaccine will last and the optimal spacing of the vaccine injections in order to give the greatest and longest-lasting protection.

The most direct way in which the JH virus could be shown to be responsible for the coldlike symptoms with which it is associated would of course be to inoculate human volunteers and show that, under controlled conditions, the JH virus produces the clinical respiratory illness. Unfortunately, we were not able to carry out such an experiment. However, it should be pointed out in this connection that the failure of the JH virus to produce the overt disease in volunteers does not necessarily mean that it is not the etiologic agent. In view of the great difficulty in isolating this agent, it is quite possible that only a few virus particles present in the total JH virus population are capable of growing in monkey kidney epithelial tissue cultures. The viruses that reproduce might be avirulent for humans. It is therefore felt that, while the human-volunteer experiment should be done, the vaccine experiments reported in this paper are direct proof that the JH virus is capable of causing coldlike symptoms in humans.

SUMMARY

1. The JH virus was found in association with an upper-respiratory outbreak in children.
2. Children given a vaccine prepared from inactivated JH virus showed an attack rate about eight times lower than that of the children receiving the placebo injections.
3. No untoward reactions were observed in 401 individuals receiving the vaccine, which was prepared from inactivated JH virus grown in monkey kidney epithelial tissue.
4. It is concluded that the JH virus is the cause of the coldlike illness in humans and that an inactivated vaccine can be prepared from this virus which protects against the overt illness due to the JH virus.

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